

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 2, line 21, with the following rewritten paragraph:

--This is illustrated by the vaccines based on M-like proteins mentioned above or disclosed in the literature, ~~i.a. in WO 98/0561~~ i.e., WO 98/01561. The binding of fibrinogen and complement factor H to M-proteins is assumed to be important for the ability of streptococci to resist phagocytosis by polymorphonuclear leucocytes.--

Please replace the paragraph beginning at page 10, line 8, with the following rewritten paragraph:

--50 mg human serum albumin (HSA, Sigma) were immobilized on a 5 ml-~~HiTrap~~ HITRAP™ NHS-activated column (Pharmacia Biotech) according to the manufacturer's instructions. Based on the absorbance at 280 nm, 65% of the HSA was estimated to be immobilized on the column.--

Please replace the paragraph beginning at page 12, line 4, with the following rewritten paragraph:

--The DNA obtained above was ligated into the plasmid vector pTYB4 [(New England Biolabs, Beverly, MA, USA (NED Inc.)], which previously had been digested with the same restriction enzymes and treated with alkaline phosphatase. After ligation (using the ReadyToGo READYTOGO™ ligation kit, Amersham), the DNA sample was electrotransformed into the *E. coli* strain ER2566 and spread on LAA-plates (Luria Bertani agar plates supplemented with ampicillin, final conc. 50 µg/ml). After incubation over night at 37°C, clones harboring plasmids with inserts were isolated and the presence of the correct insert was verified by DNA sequencing. One of the clones thereby obtained, called SFSC 1, was chosen for production of the C- terminal part of SFS.--

Please replace the paragraph beginning at page 12, line 13, with the following rewritten paragraph:

--The vector used is a part of an *E. coli* expression and purification system called IMPACT^T T7 (NEB Inc.). Briefly, following the manufacturer's instructions, the clone SFSC1 was grown at 37 °C in Luria Bertani growth medium supplemented with ampicillin (final conc. 50 µg/ml). At an optical density (OD₆₀₀) of ~ 0.6, the growth medium was supplemented with IPTG (final conc. 0.4 mM) and the growth temperature was shifted to 20°C. After incubation over night, the cells were harvested and resuspended in a buffer [20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1 mM EDTA, and 0.1 % ~~Triton X100~~ TRITONTM X100 (octylphenol ethylene oxide condensate)], lysed by freezing and thawing, and after centrifugation, the supernatant was sterile filtered and applied to a chitin column.--

Please replace the paragraph beginning at page 16, line 9, with the following rewritten paragraph:

--Both PCR amplifications were performed using ~~ReadyToGo~~ READYTOGOTM PCR beads (Amersham Pharmacia Biotech Inc) and the PCR apparatus ~~MiniCycler~~ MINICYCLERTM (MJ Research, Inc, MA, USA) using a program comprising: step 1: 95°C, 1 min; step 2: 95°C, 30 sec.; step 3: 46°C, 15 sec.; and step 4: 72°C, 2 min; repeated as 25 cycles from step 2 to step 4. After PCR amplification, the respective PCR-products were purified and digested with restriction endonucleases *NcoI* and *XhoI* and ligated into the pTYB4-vector (NEB) digested previously with the same enzymes. One µl of the respective ligation mixture was transformed into *E. coli* ER2566. After IPTG induction, *E. coli* clones expressing collagen-binding were identified by colony screening using ¹²⁵I-labeled collagen (collagen S, type I obtained from Boehringer Mannheim). Several clones of both types of constructions expressing collagen-binding were identified and further characterized. One of these clones called pSEC 2.16 harboring the PCR-fragment and originating from the PCR using OSEC 1:5 and 3:3 was chosen after DNA sequencing of the insert for production of a recombinant collagen-binding protein called protein SEC 2.16 and comprising amino acid residues 27 - 616 of SEQ. ID. NO: 4. The nucleotide sequence of this insert encoding the recombinant collagen-binding protein SEC2.16 is shown

below as SEQ. ID. NO: 19. The nucleotides shown in bold represent nucleotides originating from the vector.--

Please replace the paragraph beginning at page 18, line 10 with the following rewritten paragraph:

--Since the vector used to construct the pSEC1.18 and pSEC 2.16 is a part of the IMPACT-system (NEB), the expression and purification of protein SEC1.18 and protein SEC2.16 were performed as described for protein SFSC1 and protein FNZN. After purification, protein SEC 1.18 and protein SEC 2.16 were analysed by SDS-PAGE using the ~~Phast-System~~ PHASTSYSTEM™ (Amersham Pharmacia) and 8-25% gradient gels under reducing conditions (SDS buffer strips, boiling the samples in a sample buffer containing SDS and β -mercaptoethanol before applying the samples on the gels). The results show that the purified protein SEC 1.18 and protein SEC 2.16, approx. had relative molecular weights corresponding to their calculated molecular weights.--

Please replace the paragraph beginning at page 18, line 20 and ending at page 19, line 4 with the following rewritten paragraph:

--The purified proteins SEC 1.18 and SEC 1.16 were also analysed for their ability to bind to collagen. First, the proteins were run on an SDS-PAGE 8-25% gel (Amersham Pharmacia) under reducing conditions. After the electrophoresis was completed, the proteins were transferred (by diffusion) to a nitro-cellulose membrane. The membrane was blocked in a solution (PBS-T) containing PBS supplemented with ~~Tween-20~~ TWEENTM 20 (polyoxyethylene sorbitan monolaurate) (final concentration 0.5%) and casein 0.5% (final conc.) for 1 hr. at room temperature (RT). After washing with PBS-T (casein omitted), the membrane was transferred to a solution of PBS-T containing 125 I-labeled collagen. After 4 hrs. of incubation at RT under gentle agitation, the membrane was extensively washed using PBS-T (casein omitted) and subjected to autoradiography using ~~Biomax~~ BIOMAX™ MS (Kodak) film. The results showed that in contrast to unrelated proteins used as control (size marker proteins), both protein SEC 1.18 and protein SEC 2.16 bound collagen.--

Please replace the paragraph beginning at page 21, line 7 with the following rewritten paragraph:

--To express and purify the major part of the SclC protein, the following construction was made. The primers
OSCL2:5: 5'- CATGCCATGGACCAGCCAGCAGCACTAAAATAT-3' (SEQ. ID. NO: 25)
and OSCL3:3: 5'- CCGCTCGAGGGCTGCTTTTTGACCTGTTGGT-3' (SEQ. ID. NO: 26)
were used to PCR-amplify a DNA-fragment corresponding to amino acid 38 to amino acid 269 in protein SclC using *S. equi* subspecies *equi* 1866 chromosomal DNA as a template. PCR amplification was performed using ~~ReadyToGo~~ READYTOGOTM PCR beads (Amersham Pharmacia Biotech Inc) and the PCR apparatus ~~MiniCycler~~ MINICYCLERTM (MJ Research, Inc, MA, USA) using a program of step 1: 95°C, 1 min; step 2: 95°C 30 sec.; step 3: 50°C 15 sec.; step 4: 72°C, 1 min; repeated as 24 cycles from step 2 to step 4. After PCR amplification, the respective PCR-products were purified and digested with restriction endonucleases *NcoI* and *XhoI* and ligated into the pTYB4-vector (NEB) previously digested with the same enzymes. One µl of the respective ligation mixture was transformed into *E. coli* ER2566. *E. coli* clones harboring the *sclC* fragment were identified by DNA sequencing of the inserted fragments.—

Please replace the paragraph beginning at page 22, line 1 with the following rewritten paragraph:

--Since the vector used to construct the pSclC1 is a part of the IMPACT-system (NEB) the expression and purification of protein SCLC1 was performed as described for the other IMPACT-constructions (proteins SFSC1, FNZN, SEC 1.18 and SEC 2.16). After purification protein SCLC1 was analysed by SDS-PAGE using the ~~Phast-System~~ PHASTSYSTEMTM (Amersham Pharmacia) and 8-25% gradient gels under reducing conditions (SDS buffer strips, boiling the samples in a sample buffer containing SDS and beta-mercaptoethanol before applying the samples on the gels). The results showed that the purified protein had a relative molecular weight corresponding to its calculated molecular weight. The purified protein SCLC1 was then used to immunize mice and rabbits and was also used to screen convalescence sera from horses for measurements of antibody titers against SCLC1. This is illustrated in Example 6 below.--

Please replace the paragraph beginning at page 22, line 18 with the following rewritten paragraph:

--In a Western blot analysis, the SCLC1 protein was also run on an SDS-PAGE gel using the ~~Phast-System~~ PHASTSYSTEM™ (Amersham Pharmacia) and 8-25% gradient gel under reducing conditions. After the electrophoresis had been completed, the SCLC1 protein was diffusion blotted to a nitrocellulose-membrane. After blocking, the membrane was divided and immune serum (dilution 1:10 000 in PBS-0.05% ~~Tween-20~~ TWEENTM 20 (polyoxyethylene sorbitan monolaurate)) and pre-immune serum, respectively, were added and incubated for two hours at room temp. After washing in PBS-0.05% ~~Tween-20~~ TWEENTM 20 (polyoxyethylene sorbitan monolaurate), secondary antibodies (anti rabbit IgG, horseradish-labelled and developed in goat, Sigma) were used to detect rabbit antibodies directed against SCLC1. The result showed that the immune serum detected the immobilized SCLC1 protein efficiently, while no detection was seen using the pre-immune serum.--